

SEQUENCE LISTING



<110> Miller, Brian S.
Shetty, Jayarama K.

<120> Modified Forms of Pullulanase

<130> GC396-2

<140> 09/262,126

<141> 1999-03-03

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 Pro Trp Ser Leu Trp Met Trp Pro Lys Asp Gly Gly Gly Ala Glu Tyr
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 Asp Phe Asn Gln Pro Ala Asp Ser Phe Gly Ala Val Ala Ser Ala Asp
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 Ile Pro Gly Asn Pro Ser Gln Val Gly Ile Ile Val Arg Thr Gln Asp
 85 90 95
 Trp Thr Lys Asp Val Ser Ala Asp Arg Tyr Ile Asp Leu Ser Lys Gly
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 Asn Glu Val Trp Leu Val Glu Gly Asn Ser Gln Ile Phe Tyr Asn Glu
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 Lys Asp Ala Glu Asp Ala Ala Lys Pro Ala Val Ser Asn Ala Tyr Leu
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 Asp Ala Ser Asn Gln Val Leu Val Lys Leu Ser Gln Pro Leu Thr Leu
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 Gly Glu Gly Xaa Ser Gly Phe Thr Val His Asp Asp Thr Ala Asn Lys
 165 170 175
 Asp Ile Pro Val Thr Ser Val Lys Asp Ala Ser Leu Gly Gln Asp Val

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
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| Thr | Ala | Val | Leu | Ala | Gly | Thr | Phe | Gln | His | Ile | Phe | Gly | Gly | Ser | Asp |
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| Trp | Ala | Pro | Asp | Asn | His | Ser | Thr | Leu | Leu | Lys | Lys | Val | Thr | Asn | Asn |
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| Leu | Tyr | Gln | Phe | Ser | Gly | Asp | Leu | Pro | Glu | Gly | Asn | Tyr | Gln | Tyr | Lys |
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| Val | Ala | Leu | Asn | Asp | Ser | Trp | Asn | Asn | Ser | Tyr | Pro | Ser | Asp | Asn | Ile |
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| Asn | Leu | Thr | Val | Pro | Ala | Gly | Gly | Ala | His | Val | Thr | Phe | Ser | Tyr | Ile |
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| Pro | Ser | Thr | His | Ala | Val | Tyr | Asp | Thr | Ile | Asn | Asn | Pro | Asn | Ala | Asp |
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| Leu | Gln | Val | Glu | Ser | Gly | Val | Lys | Thr | Asp | Leu | Val | Thr | Val | Thr | Leu |
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| Gly | Glu | Asp | Pro | Asp | Val | Ser | His | Thr | Leu | Ser | Ile | Gln | Thr | Asp | Gly |
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| Tyr | Gln | Ala | Lys | Gln | Val | Ile | Pro | Arg | Asn | Val | Leu | Asn | Ser | Ser | Gln |
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| Tyr | Tyr | Tyr | Ser | Gly | Asp | Asp | Leu | Gly | Asn | Thr | Tyr | Thr | Gln | Lys | Ala |
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| Thr | Thr | Phe | Lys | Val | Trp | Ala | Pro | Thr | Ser | Thr | Gln | Val | Asn | Val | Leu |
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| Leu | Tyr | Asp | Ser | Ala | Thr | Gly | Ser | Val | Thr | Lys | Ile | Val | Pro | Met | Thr |
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| Ala | Ser | Gly | His | Gly | Val | Trp | Glu | Ala | Thr | Val | Asn | Gln | Asn | Leu | Glu |
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| Asn | Trp | Tyr | Tyr | Met | Tyr | Glu | Val | Thr | Gly | Gln | Gly | Ser | Thr | Arg | Thr |
| | | | | 405 | | | | | 410 | | | | | 415 | |
| Ala | Val | Asp | Pro | Tyr | Ala | Thr | Ala | Ile | Ala | Pro | Asn | Gly | Thr | Arg | Gly |
| | | | 420 | | | | | 425 | | | | | 430 | | |
| Met | Ile | Val | Asp | Leu | Ala | Lys | Thr | Asp | Pro | Ala | Gly | Trp | Asn | Ser | Asp |
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| Lys | His | Ile | Thr | Pro | Lys | Asn | Ile | Glu | Asp | Glu | Val | Ile | Tyr | Glu | Met |
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| Asp | Val | Arg | Asp | Phe | Ser | Ile | Asp | Pro | Asn | Ser | Gly | Met | Lys | Asn | Lys |
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| Gly | Lys | Tyr | Leu | Ala | Leu | Thr | Glu | Lys | Gly | Thr | Lys | Gly | Pro | Asp | Asn |
| | | | | 485 | | | | | 490 | | | | | 495 | |
| Val | Lys | Thr | Gly | Ile | Asp | Ser | Leu | Lys | Gln | Leu | Gly | Ile | Thr | His | Val |
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| Gln | Leu | Met | Pro | Val | Phe | Ala | Ser | Asn | Ser | Val | Asp | Glu | Thr | Asp | Pro |
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| Thr | Gln | Asp | Asn | Trp | Gly | Tyr | Asp | Pro | Arg | Asn | Tyr | Asp | Val | Pro | Glu |
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| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | Ile | Asp | Gly | Phe | Arg | Phe | Asp | Leu | Met | Ala | Leu | Leu | Gly | Lys | Asp |
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| Thr | Met | Ser | Lys | Ala | Ala | Ser | Glu | Leu | His | Ala | Ile | Asn | Pro | Gly | Ile |
| | | | 660 | | | | | 665 | | | | | 670 | | |
| Ala | Leu | Tyr | Gly | Glu | Pro | Trp | Thr | Gly | Gly | Thr | Ser | Ala | Leu | Pro | Asp |
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| Asp | Gln | Leu | Leu | Thr | Lys | Gly | Ala | Gln | Lys | Gly | Met | Gly | Val | Ala | Val |
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| Phe | Asn | Asp | Asn | Leu | Arg | Asn | Ala | Leu | Asp | Gly | Asn | Val | Phe | Asp | Ser |
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| Lys | Asn | Gly | Val | Glu | Gly | Ser | Ile | Asn | Asp | Phe | Thr | Ser | Ser | Pro | Gly |
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| Glu | Thr | Ile | Asn | Tyr | Val | Thr | Ser | His | Asp | Asn | Tyr | Thr | Leu | Trp | Asp |
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| Lys | Ile | Ala | Leu | Ser | Asn | Pro | Asn | Asp | Ser | Glu | Ala | Asp | Arg | Ile | Lys |
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| Met | Asp | Glu | Leu | Ala | Gln | Ala | Val | Val | Met | Thr | Ser | Gln | Gly | Val | Pro |
| 785 | | | | | 790 | | | | | 795 | | | | | 800 |
| Phe | Met | Gln | Gly | Gly | Glu | Glu | Met | Leu | Arg | Xaa | Lys | Gly | Gly | Asn | Asp |
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| Asn | Ser | Tyr | Asn | Ala | Gly | Asp | Ala | Val | Asn | Glu | Phe | Asp | Trp | Ser | Arg |
| | | | 820 | | | | | 825 | | | | | 830 | | |
| Lys | Ala | Gln | Tyr | Pro | Asp | Val | Phe | Asn | Tyr | Tyr | Ser | Gly | Leu | Ile | His |
| | 835 | | | | | | 840 | | | | | 845 | | | |
| Leu | Arg | Leu | Asp | His | Pro | Ala | Phe | Arg | Met | Thr | Thr | Ala | Asn | Glu | Ile |
| | 850 | | | | | 855 | | | | | 860 | | | | |
| Asn | Ser | His | Leu | Gln | Phe | Leu | Asn | Ser | Pro | Glu | Asn | Thr | Val | Ala | Tyr |
| 865 | | | | | 870 | | | | | 875 | | | | | 880 |
| Glu | Leu | Thr | Asp | His | Val | Asn | Lys | Asp | Lys | Trp | Gly | Asn | Ile | Ile | Val |
| | | | 885 | | | | | | 890 | | | | | 895 | |
| Val | Tyr | Asn | Pro | Asn | Lys | Thr | Val | Ala | Thr | Ile | Asn | Leu | Pro | Ser | Gly |
| | | 900 | | | | | | 905 | | | | | 910 | | |
| Lys | Trp | Ala | Ile | Asn | Ala | Thr | Ser | Gly | Lys | Val | Gly | Glu | Ser | Thr | Leu |
| | 915 | | | | | | 920 | | | | | 925 | | | |
| Gly | Gln | Ala | Glu | Gly | Ser | Val | Gln | Val | Pro | Gly | Ile | Ser | Met | Met | Ile |
| | 930 | | | | | 935 | | | | | 940 | | | | |
| Leu | His | Gln | Glu | Val | Ser | Pro | Asp | His | Gly | Lys | Lys | Xaa | Lys | | |
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<213> B. subtilis

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| Ile | Ile | Thr | Val | Leu | Ile | Pro | Ala | Glu | Gln | Lys | Glu | Ile | Met | Thr | Pro |
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| Pro | Phe | Arg | Leu | Glu | Thr | Glu | Ile | Thr | Asp | Phe | Pro | Leu | Ala | Val | Arg |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Glu | Glu | Tyr | Ser | Leu | Glu | Ala | Lys | Tyr | Lys | Tyr | Val | Cys | Val | Ser | Asp |
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| His | Pro | Val | Thr | Phe | Gly | Lys | Ile | His | Cys | Val | Arg | Ala | Ser | Ser | Gly |
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| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| His | Lys | Thr | Asp | Leu | Gln | Ile | Gly | Ala | Val | Ile | Arg | Thr | Ala | Ala | Phe | |
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| Asp | Asp | Glu | Phe | Tyr | Tyr | Asp | Gly | Glu | Leu | Gly | Ala | Val | Tyr | Thr | Ala | |
| | | | 100 | | | | | 105 | | | | | 110 | | | |
| Asp | His | Thr | Val | Phe | Lys | Val | Trp | Ala | Pro | Ala | Ala | Thr | Ser | Ala | Ala | |
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| Val | Lys | Leu | Ser | His | Pro | Asn | Lys | Ser | Gly | Arg | Thr | Phe | Gln | Met | Thr | |
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| Thr | Val | Asp | Gln | Tyr | Ala | Lys | Ala | Val | Thr | Val | Asn | Gly | Glu | Lys | Gly | |
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| Val | Val | Leu | Arg | Pro | Asp | Gln | Met | Lys | Trp | Thr | Ala | Pro | Leu | Lys | Pro | |
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| Phe | Ser | His | Pro | Val | Asp | Ala | Val | Ile | Tyr | Glu | Thr | His | Leu | Arg | Asp | |
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| Phe | Ser | Ile | His | Glu | Asn | Ser | Gly | Met | Ile | Asn | Lys | Gly | Lys | Tyr | Leu | |
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| Ala | Leu | Thr | Glu | Thr | Asp | Thr | Gln | Thr | Ala | Asn | Gly | Ser | Ser | Ser | Gly | |
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| Leu | Ala | Tyr | Val | Lys | Glu | Leu | Gly | Val | Thr | His | Val | Glu | Leu | Leu | Pro | |
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| Val | Asn | Asp | Phe | Ala | Gly | Val | Asp | Glu | Glu | Lys | Pro | Leu | Asp | Ala | Tyr | |
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| Asn | Trp | Gly | Tyr | Asn | Pro | Leu | His | Phe | Phe | Ala | Pro | Glu | Gly | Ser | Tyr | |
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| Ala | Ser | Asn | Pro | His | Asp | Pro | Gln | Thr | Arg | Lys | Thr | Glu | Leu | Lys | Gln | |
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| Met | Ile | Asn | Thr | Leu | His | Gln | His | Gly | Leu | Arg | Val | Ile | Leu | Asp | Val | |
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| Val | Phe | Asn | His | Val | Tyr | Lys | Arg | Glu | Asn | Ser | Pro | Phe | Glu | Lys | Thr | |
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| Val | Pro | Gly | Tyr | Phe | Phe | Arg | His | Asp | Glu | Cys | Gly | Met | Pro | Ser | Asn | |
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| Gly | Thr | Gly | Val | Gly | Asn | Asp | Ile | Ala | Ser | Glu | Arg | Arg | Met | Ala | Arg | |
| | | | 370 | | | 375 | | | | | | | 380 | | | |
| Lys | Phe | Ile | Ala | Asp | Cys | Val | Val | Tyr | Trp | Leu | Glu | Glu | Tyr | Asn | Val | |
| 385 | | | | | 390 | | | | | | 395 | | | | 400 | |
| Asp | Gly | Phe | Arg | Phe | Asp | Leu | Leu | Gly | Ile | Leu | Asp | Ile | Asp | Thr | Val | |
| | | | | 405 | | | | | 410 | | | | | 415 | | |
| Leu | Tyr | Met | Lys | Glu | Lys | Ala | Thr | Lys | Ala | Lys | Pro | Gly | Ile | Leu | Leu | |
| | | | 420 | | | | | 425 | | | | | | 430 | | |
| Phe | Gly | Glu | Gly | Trp | Asp | Leu | Ala | Thr | Pro | Leu | Pro | His | Glu | Gln | Lys | |
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| Ala | Ala | Leu | Ala | Asn | Ala | Pro | Arg | Met | Pro | Gly | Ile | Gly | Phe | Phe | Asn | |
| | | | 450 | | | 455 | | | | | | 460 | | | | |
| Asp | Met | Phe | Arg | Asp | Ala | Val | Lys | Gly | Asn | Thr | Phe | His | Leu | Lys | Ala | |
| 465 | | | | | 470 | | | | | 475 | | | | | 480 | |
| Thr | Gly | Phe | Ala | Leu | Gly | Asn | Gly | Glu | Ser | Ala | Gln | Ala | Val | Met | His | |
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| Gly | Ile | Ala | Gly | Ser | Ser | Gly | Trp | Lys | Ala | Leu | Ala | Pro | Ile | Val | Pro | |
| | | | 500 | | | | | 505 | | | | | | 510 | | |
| Glu | Pro | Ser | Gln | Ser | Ile | Asn | Tyr | Val | Glu | Ser | His | Asp | Asn | His | Thr | |
| | | | 515 | | | | 520 | | | | | | 525 | | | |
| Phe | Trp | Asp | Lys | Met | Ser | Phe | Ala | Leu | Pro | Gln | Glu | Asn | Asp | Ser | Arg | |

| | | | | |
|-------------------------|-------------------------|---------------------|--|-----|
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| Lys Arg Ser Arg Gln Arg | Leu Ala Val Ala Ile Ile | Leu Leu Ala Gln | | |
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| Gly Val Pro Phe Ile His | Ser Gly Gln Glu Phe Phe | Arg Thr Lys Gln | | |
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| Gly Val Glu Asn Ser Tyr | Gln Ser Ser Asp Ser Ile | Asn Gln Leu Asp | | |
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| Trp Asp Arg Arg Glu Thr | Phe Lys Glu Asp Val His | Tyr Ile Arg Arg | | |
| | 595 | 600 | | 605 |
| Leu Ile Ser Leu Arg Lys | Ala His Pro Ala Phe | Arg Leu Arg Ser Ala | | |
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| Ala Asp Ile Gln Arg His | Leu Glu Cys Leu Thr | Leu Lys Glu His Leu | | |
| 625 | | 630 | | 640 |
| Ile Ala Tyr Arg Leu Tyr | Asp Leu Asp Glu Val | Asp Glu Trp Lys Asp | | |
| | 645 | 650 | | 655 |
| Ile Ile Val Ile His His | Ala Ser Pro Asp Ser | Val Glu Trp Arg Leu | | |
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| Pro Asn Asp Ile Pro Tyr | Arg Leu Leu Cys Asp | Pro Ser Gly Phe Gln | | |
| | 675 | 680 | | 685 |
| Glu Asp Pro Thr Glu Ile | Lys Lys Thr Val Ala | Val Asn Gly Ile Gly | | |
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| Thr Val Ile Leu Tyr Leu | Ala Ser Asp Leu Lys | Ser Phe Ala | | |
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| |
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| 35 40 45 |
| Ala Val Pro Gly Glu Ala Val Thr Ala Val Glu Asn Gln Ala Val Ile |
| 50 55 60 |
| His Leu Val Asp Ile Ala Gly Ile Thr Ser Ser Ser Ala Ala Asp Tyr |
| 65 70 75 80 |
| Ser Ser Lys Asn Leu Tyr Leu Trp Asn Asn Glu Thr Cys Asp Ala Leu |
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| Ser Ala Pro Val Ala Asp Trp Asn Asp Val Ser Thr Thr Pro Ser Gly |
| 100 105 110 |
| Ser Asp Lys Tyr Gly Pro Tyr Trp Val Ile Pro Leu Asn Lys Glu Ser |
| 115 120 125 |
| Gly Cys Ile Asn Val Ile Val Arg Asp Gly Thr Asp Lys Leu Ile Asp |
| 130 135 140 |
| Ser Asp Leu Arg Val Ala Phe Gly Asp Phe Thr Asp Arg Thr Val Ser |
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| Val Ile Ala Gly Asn Ser Ala Val Tyr Asp Ser Arg Ala Asp Ala Phe |
| 165 170 175 |
| Arg Ala Ala Phe Gly Val Ala Leu Ala Glu Ala His Trp Val Asp Lys |
| 180 185 190 |
| Asn Thr Leu Leu Trp Pro Gly Gly Gln Asp Lys Pro Ile Val Arg Leu |
| 195 200 205 |
| Tyr Tyr Ser His Ser Ser Lys Val Ala Ala Asp Gly Glu Gly Lys Phe |

| | | |
|-------------------------|---------------------|---------------------|
| 210 | 215 | 220 |
| Thr Asp Arg Tyr Leu Lys | Leu Thr Pro Thr Thr | Val Ser Gln Gln Val |
| 225 | 230 | 235 |
| Ser Met Arg Phe Pro His | Leu Ser Ser Tyr Ala | Ala Phe Lys Leu Pro |
| 245 | 250 | 255 |
| Asp Asn Ala Asn Val Asp | Glu Leu Leu Gln Gly | Glu Thr Val Ala Ile |
| 260 | 265 | 270 |
| Ala Ala Ala Glu Asp Gly | Ile Leu Ile Ser Ala | Thr Gln Val Gln Thr |
| 275 | 280 | 285 |
| Ala Gly Val Leu Asp Asp | Ala Tyr Ala Glu Ala | Ala Glu Ala Leu Ser |
| 290 | 295 | 300 |
| Tyr Gly Ala Gln Leu Ala | Asp Gly Gly Val Thr | Phe Arg Val Trp Ala |
| 305 | 310 | 315 |
| Pro Thr Ala Gln Gln Val | Asp Val Val Val Tyr | Ser Ala Asp Lys Lys |
| 325 | 330 | 335 |
| Val Ile Gly Ser His Pro | Met Thr Arg Asp Ser | Ala Ser Gly Ala Trp |
| 340 | 345 | 350 |
| Ser Trp Gln Gly Gly Ser | Asp Leu Lys Gly Ala | Phe Tyr Arg Tyr Ala |
| 355 | 360 | 365 |
| Met Thr Val Tyr His Pro | Gln Ser Arg Lys Val | Glu Gln Tyr Glu Val |
| 370 | 375 | 380 |
| Thr Asp Pro Tyr Ala His | Ser Leu Ser Thr Asn | Ser Glu Tyr Ser Gln |
| 385 | 390 | 395 |
| Val Val Asp Leu Asn Asp | Ser Ala Leu Lys Pro | Asp Gly Trp Asp Asn |
| 405 | 410 | 415 |
| Leu Thr Met Pro His Ala | Gln Lys Thr Lys Ala | Asp Leu Ala Lys Met |
| 420 | 425 | 430 |
| Thr Ile His Glu Ser His | Ile Arg Asp Leu Ser | Ala Trp Asp Gln Thr |
| 435 | 440 | 445 |
| Val Pro Ala Glu Leu Arg | Gly Lys Tyr Leu Ala | Leu Thr Ala Gly Asp |
| 450 | 455 | 460 |
| Ser Asn Met Val Gln His | Leu Lys Thr Leu Ser | Ala Ser Gly Val Thr |
| 465 | 470 | 475 |
| His Val Glu Leu Leu Pro | Val Phe Asp Leu Ala | Thr Val Asn Glu Phe |
| 485 | 490 | 495 |
| Ser Asp Lys Val Ala Asp | Ile Gln Gln Pro Phe | Ser Arg Leu Cys Glu |
| 500 | 505 | 510 |
| Val Asn Ser Ala Val Lys | Ser Ser Glu Phe Ala | Gly Tyr Cys Asp Ser |
| 515 | 520 | 525 |
| Gly Ser Thr Val Glu Glu | Val Leu Asn Gln Leu | Lys Gln Ser Asp Ser |
| 530 | 535 | 540 |
| Gln Asp Asn Pro Gln Val | Gln Ala Leu Asn Thr | Leu Val Ala Gln Thr |
| 545 | 550 | 555 |
| Asp Ser Tyr Asn Trp Gly | Tyr Asp Pro Phe His | Tyr Thr Val Pro Glu |
| 565 | 570 | 575 |
| Gly Ser Tyr Ala Thr Asp | Pro Glu Gly Thr Thr | Arg Ile Lys Glu Phe |
| 580 | 585 | 590 |
| Arg Thr Met Ile Gln Ala | Ile Lys Gln Asp Leu | Gly Met Asn Val Ile |
| 595 | 600 | 605 |
| Met Asp Val Val Tyr Asn | His Thr Asn Ala Ala | Gly Pro Thr Asp Arg |
| 610 | 615 | 620 |
| Thr Ser Val Leu Asp Lys | Ile Val Pro Trp Tyr | Tyr Gln Arg Leu Asn |
| 625 | 630 | 635 |
| Glu Thr Thr Gly Ser Val | Glu Ser Ala Thr Cys | Cys Ser Asp Ser Ala |
| 645 | 650 | 655 |
| Pro Glu His Arg Met Phe | Ala Lys Leu Ile Ala | Asp Ser Leu Ala Val |
| 660 | 665 | 670 |

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